

Lakes, Labs and Learning: How an Environmental DNA Citizen Science Project Makes Sense for High School Students, Researchers and Environmental Managers

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Abstract

Citizen science (CS) is a fairly new concept where ordinary citizens contribute to scientific research. At the Natural History Museum of Denmark, a successful pilot CS project was followed up by an actual full-scale project. As part of their curricula, high-school students learned and tested newly developed environmental DNA (eDNA) working methods on lake water to document which fish, amphibians, insects, crayfish and mammals were living in the lakes. Initially CS was met with scepticism by researchers but now CS is becoming more visible among researchers as well as environmental managers and STEM educators.

Keywords: Environmental DNA; citizen science; environmental monitoring; outreach; high schools; science education; molecular methods

Background

The context of this article is based on the molecular biological research carried out at the Natural History Museum of Denmark (NHMD), research which later became operationalized into the citizen science project *DNA og Liv* [DNA and Life], one of the museum's citizen science projects targeting high school students and teachers.

Since 2010 the museum's Centre for GeoGenetics has been in the forefront in international research when it comes to use of ancient as well as modern environmental DNA (eDNA). DNA from ancient environments has, e.g., revealed causes of Ice Age megafauna extinctions (Lorenzen et al., 2011), survival of conifers in Ice Age Scandinavia (Parducci et al., 2012) and showed that a textbook theory of how humans populated America was biologically unviable (Pedersen et al., 2016). From modern environments like Danish freshwater lakes (Thomsen et al., 2012), deep subarctic waters (Thomsen et al., 2016) and high-saline waters of the Persian Gulf (Sigsgaard et al., 2016) species composition of organisms can be detected using relatively simple DNA methods.

Through a collaboration between the museum's researchers, the public engagement department, a number of high school classes, and with funding from the Lundbeck Foundation a pilot study was carried out using the eDNA method on Danish lakes.

This article describes the initial idea behind the project, how the project was designed and implemented and eventually what came out of it, namely that students from local high schools around the country collected and analyzed samples from local waters with the educational benefit that they got hands on experience both in the field and in the lab, using an approach that integrates science, mathematics and technology, and learning how scientists work.

Another potential outcome of the project relates to natural resource management. It is often claimed by advocates of the eDNA method that it will make environmental monitoring easier and cheaper compared to the more traditional methods, which take a lot of logistics to carry out. Environmental DNA can provide both scientists and environmental managers with valuable and credible data.

The Concept and Use of Environmental DNA (eDNA)

All living things release DNA into their environment. This can be from faeces, urine, eggs, skin cells, hair, bones, tissue or teeth. It is the physical, biological and chemical conditions which determine how fast DNA will decompose into fragments or be preserved in an environment.

High temperature and moist conditions like in the tropics are bad for preservation. Likewise, low pH in, e.g., bogs is also bad for preservation. In other parts of the world, like in the Arctic, low temperatures and permafrost are ideal conditions for extracting useful ancient environmental DNA.

In aqueous environments DNA is degraded via three factors (Barnes et al., 2014):

- The condition of the DNA (length, intra- or extracellular);
- Abiotic factors (e.g. temperature, UV-radiation);
- Biotic factors (microbes and extracellular enzymes).

Of particular importance is the presence of microbes and enzymes. The degradation of the eDNA happens exponentially over time and can happen from a few hours up to a month. Furthermore, Salter (2018) showed that phosphorous limitation can have a substantial effect on degradation time in sea water.

Because of the presence of DNA in aqueous systems it has been suggested that eDNA-methods can supplement or even replace traditional environmental monitoring methods. Kelly et al., (2014) mention two strong advantages over conventional techniques: increased sensitivity and reduced cost. DNA-based detection outperforms other common biological survey techniques in terms of number of species detected and does so with non-invasive sampling.

Apart from detecting the presence of species, a connection between biomass and eDNA concentration has been found in fresh water systems as well as in marine environments. Furthermore, genetic variation within a population can be seen in the eDNA; this was demonstrated in a study on whale sharks in the Persian Gulf and used to determine the population size of the sharks (Sigsgaard et al., 2016).

Citizen Science: Seriously - Can Citizens Really Do Science?

Citizen science is a relatively new way to involve the public in science outreach. In 2013 the European Commission published a "Green Paper on Citizen Science" (European Commission, 2013) which concluded that "Different definitions can be found for Citizen Science, where some take up more traditional aspects, understanding Citizen Science as an approach, which involves volunteers from the general public in scientific investigations during data collection and analysis. Others define it more broadly, as the public participating in scientific research, which includes also scientific activities like the asking of questions, formulation of hypotheses, interpretation of results. Current discussions around the definition of citizen science not only focus on the scope of activities but also what to understand under "volunteers" and how to composite citizen science teams. What we cannot find is one generally accepted definition of citizen science yet."

Rick Bonney, an American ornithologist, had a more practical definition (Bonney et al., 2009) as he defined CS as "projects in which nonscientists, such as amateur birdwatchers, voluntarily contribute scientific data."

Purpose of the Project *DNA og Liv* [DNA and Life]

Based on the new eDNA-method (Thomsen et al., 2012) the NHMD started developing the educational initiative the same year the method was published in 2012. With the experience from a one-year pilot citizen science project carried out at NHMD involving trial and error lake sampling and subsequent laboratory work, the full-scale, two-year citizen science initiative *DNA og Liv* was offered to high school teachers and students from 2015. Originally the aim of the project was to involve students in the newest research carried out at the museum and offer them something related to their everyday lives – not just standard samples from a prefabricated kit but samples from their own world which they could use in their local context. In the beginning the project was not conceived as a citizen science project but during the process it became one. And initially researchers were not involved – on the contrary: They were sceptical. Through hard work the researcher involvement slowly made its way into the project as another ongoing NHMD citizen science project–the national fish atlas project–became involved in *DNA og Liv*.

One important aspect which gradually dawned on the museum staff was the fact that a narrative about a researcher who is sitting waiting for your results is hugely motivating for the students. They are used to their reports being ditched after exams – but here someone actually wants their work. As of 2017, 300-400 high school classes from all parts of Denmark have participated in the project.

DNA og Liv is one of several citizen science projects at the NHMD and it is important for the museum because as a national museum it has obligations to cover all of Denmark in its outreach activities as well as disseminate the research results from the museum's scientists. Furthermore, the project naturally covers the curricula set up by the Ministry of Education and has been developed in collaboration with high school teachers

From Lake to Lab

The basic idea about *DNA og Liv* is to move students from class rooms to lakes to labs and see if they can detect traces of DNA from the animals living in the lakes. In the first step the students choose a local location which they find relevant and interesting, preferably in springtime when life is returning after a cold winter. Sampling is done from the water column and care should be taken to get a clean sample and not include silt or mud from the lake bottom. A couple of hundred milliliters is enough and after sampling, ethanol is added in order to stop DNA degradation.

In step 2 the samples are sent to the NHMD laboratory in Copenhagen where they are prepared by museum technicians. First the samples are centrifuged at high rotation speed so that DNA will accumulate at the bottom of the test tube. Subsequently the samples are purified and DNA from the lake organisms is isolated using a modified protocol from the Qiagen blood and tissue sample kit (Qiagen, 2018).

In the third step students get to work with their own samples in NHMD's laboratories. The students then amplify DNA from specific targeted species.

As DNA is invisible the students will have to use molecular "fish hooks"—called primers—to spot the DNA. Primers are sequences of DNA which target specific species like eel or pike. In the set-up the students work with three kinds of samples: two known control samples and one unknown – the student's own sample. Using this approach there is a good chance of making a credible identification of the species in the student's sample.



Figure 1. The environmental DNA-method is based on the fact that all living things release DNA into their environment. This makes it possible to detect both rare, common and invasive species of a broad range of organisms in a single water sample from a lake or pond. (Photo: Christian Mailand, DNHM).

When looking at the results and comparing them to databases the students view three different curves, which represent a positive and a negative control sample as well as their own lake sample. The control samples are included as quality assurance for both students and researchers to make sure that the lab work has been carried out satisfactorily. Occasionally the students experience the frustration, which scientists also experience now and then when their preparatory work has been flawed and the result doesn't make sense. Other times they don't detect DNA – which doesn't necessarily mean that the lake is lifeless, just that they didn't catch the DNA. On the other hand, euphoria can set in if the students find a species which they didn't (or did) expect to find in the lake!



Figure 2. DNA og Liv moves students from classrooms to lakes to labs. So far more than 300 high school classes from all parts of Denmark have participated in the project. (Photo: Anders Peter Schultz, DNHM).



Figure 3. Students contribute to the museum scientists' research projects which enables testing at a much larger scale than otherwise possible. The collaboration with scientists is highly motivating for the students. (Photo: Christian Mailand, DNHM)

What Came Out of It?

Now that *DNA og Liv* has been running for three years some conclusions can be drawn from the different participants and their testimonials. A few statements from the users – high school teachers and students – of this educational initiative are expressed in the following quotes taken from the project evaluation:

"This project is different and to a high degree something which we can't do ourselves. Generally, eDNA is interesting as an educational opportunity in biotechnology and molecular biology – not least when we incorporate it with ecology and biodiversity. I think it gives ownership to the students when they have seen the lake... and they are more motivated when it is their own data." (High school teacher and author of text books for high school biology)

"It is great as a student to contribute to research which means something to you professionals. It makes a big difference that you are so interested in it. Likewise for my own project. My class analyzed a water sample from our own local area. It means something that it was from a place we had chosen ourselves. I live next to the creek – it is a place most people in town know." (Last year high school student)

"The project gives us – the students – a good opportunity to contribute to research. It gives us a large practical outcome which we can't get in high school. At the same time, we build up a greater biological understanding." (Last year high school student)

From the museum's view the outreach department as well as the museum itself have lived up to their institutional obligations and given several thousand students a valuable research experience. It was highly motivating for the students when they arrived in the laboratory to see that although the museum is physically situated in classical historical buildings they were going to work in a dedicated, modern state of the art laboratory where, e.g., there were enough pipettes for everyone – in contrast to another reality in many high school labs. And for the NHMD itself it has learned quite a lot about how to produce and implement citizen science projects for high schools.

Naturally the project also encountered challenges. Because the method was so new they had to modify it along the way as in the beginning they didn't get any DNA in their samples. By introducing filtering of water they could process more lake water on location and catch DNA in the filters.

The end users of the collected data—the scientists—are satisfied as well. Peter Rask Møller, Associate Professor and curator of fishes at the Natural History Museum of Denmark, said that:

"In recent years we have used citizen science in ongoing national fish atlas projects, where anglers and fishermen report their catch in order to get a detailed overview of the status of our fish fauna. All data are welcome – and it has turned out that some species are almost exclusively mapped by anglers and completely missed out by the official governmental fish surveys.

"The last couple of years we have also had a close cooperation with our high-school education program – *DNA og Liv*. The students have helped testing advanced molecular methods for detection of aquatic animals and I have been surprised how well it has worked out. The students have really contributed to our research and we have been able to test the systems at much larger scale than otherwise possible.

"In both projects citizen science has worked out extremely well, thanks to the citizens but also thanks to the professional biologists hired to manage the contact to anglers and students and to quality check the many data."



Figure 4. As DNA is invisible the students have to use molecular "fish hooks" – called primers – to spot the DNA. The hands-on experience both in the field and in the lab helps open the black box of molecular and scientific processes to the participating students. (Photo: Christian Mailand, DNHM).

Resources

Making and sending sampling kits to high schools around Denmark doesn't come cheaply – neither does equipping a dedicated DNA laboratory for the project as well as analyzing numerous samples. Without a considerable donation from the Lundbeck Foundation the NHMD would not have had the financial muscles to carry out *DNA og Liv*. The Foundation's donation covered setting up a new laboratory dedicated to the project as well as running costs for the first two years. Two permanent staff from the museum have

spent many hours with the students in the laboratory and with the follow up discussions about their results. The high schools pay for each student's expenses in the lab (reagents).

***DNA og Liv* and Citizen Science in a Broader Perspective**

The *DNA og Liv* project has been fortunate in securing a rather large amount of funding from the Danish Lundbeck Foundation. Without this donation, e.g., establishing of the state-of-the-art laboratory would not have been possible.

The *DNA og Liv* project has served as a case study in how museum outreach staff can design, implement and get valuable feed-back for future citizen science projects – and not only projects targeting high schools but the general public in a broader sense. However, it would be a mistake to assume that because it is citizens who do the collecting it is a free ride for the museum staff. Human as well as financial resources for outreach, teaching and research have to be allocated.

According to feedback from both high school teachers and students the *DNA og Liv* project had a big impact on their understanding of laboratory procedures and DNA analysis. The impact of this particular project was consequently limited to high school people.

However, other citizen science projects have content which is appealing to a broader audience. An example of this is a citizen science project with its first season in 2017 and now running from May to September 2018. This project is targeting both school classes and Danish families. Called Myrejagten ("The Ant Hunt") (Statens Naturhistoriske Museum [Natural History Museum of Denmark], 2018), the project is a modified version of Rob Dunn's "School of Ants" citizen science project (Dunn, 2018). Myrejagten's focus is on mapping and studying ant species in Denmark and their diets in order to observe climate change and the environment. The project has been supported by external funding from four private Danish foundations.

Participants buy a project kit containing 8 grams of sugar, a bit of cooking oil, 6 plastic sample bags, 1 ant identification key and 17 other items. The kit costs about 7 USD. The intention is that the participants put out different baits, collect the baits and count the ants. Ants and baits are then sent to the NHMD where researchers will determine the species and compare them with species around the world.

The project is quite media-friendly as most people have some kind of reaction to ants, can involve the whole family and it has been featured in regional TV news. The project got extra publicity when a class from a local primary school next to the NHMD discovered a new ant species in the museum's Botanical Garden.

What's Next?

Based on the experience from *DNA og Liv* the bigger and more ambitious project REAL SCIENCE started up in 2017 and will be running for two years, funded again by the Lundbeck Foundation. REAL SCIENCE is a kind of umbrella covering teaching, engagement, research and development activities as well as CS at a high professional level. *DNA og Liv* is one of them. The overall aim of REAL SCIENCE is to make a total inventory of all amphibians living in Denmark.

A good signal that the method is getting more accepted is that the project will be collaborating with the Danish Environmental Protection Agency. The DEPA-project will focus on the sea in an attempt to map invasive species in all Danish waters.

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